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Possible Ameliorative Effect of Zinc in Inhibiting the Toxicity of Tak as Organochlorine Pesticide on Oreochromis Niloticus.

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Abstract: *The influence of sub lethal concentrations of Zinc (4.8 and 9.6 mg/l)and the experimental organochlorine pesticide Tak (1.5 and 3 mg/l)individually and in mixture on Nile tilapia (Oreochromis niloticus)for fifteen days were investigated .The results revealed a significant increase ((P≤0.05)in liver residual Zinc and Tak than muscle tissue of Oreochromis niloticus , accompanied by a disturbance in fish physiological status with significant increase, in serum glucose level , cortisol hormone ,cholesterol , triglycerides , urea , creatinine , AST and ALT activities. The results also demonstrated synergistic effect of Zinc and Tak on Oreochromis niloticus fish exposed to the different studied sub lethal concentrations for 15 days.*
Keywords: *O.niloticus , Zinc, Tak , serum constituents , Bioaccumulation.*

I. Introduction

Pollution of environment is the state of contamination of the various components of our natural environment caused by various economic and other human activities, at a pace that the natural forces fail to diffuse the effects of this contamination of these components of the environment viz. air, water, soil, serenity, temperature etc. The acts polluting the environment are legion, but the resultant effects or pollution may be classified into following categories: Air pollution, Noise Pollution ,Water pollution , Thermal pollution Land or soil pollution , Radio-active pollution , Solid Waste Pollution , Germ-plasm pollution or pollution of Biological wealth **[1].** Environmental pollution by toxicants has become one of the most important problems in the world **[2].** The heavy metal and pesticide contamination of aquatic system has attracted the attention of researchers all over the world **[3]** .The essential heavy metals exert biochemical and physiological functions in plants and animals. They are important constituents of several key enzymes and play important roles in various oxidation-reduction reactions such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), olybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) **[4].** The extensive uses of pesticides in Egypt contribute seriously in the contamination of the environment, especially the aquatic ecosystem which serves as reservoir for tremendous quantities of these foreign Organic chemicals **[5]** . Zn is a main element for every organism, but in high amount it is toxic for these organism and leading to physiological disorders. It gets residue in different parts or organs of fish and by food chain reaching to the body of human being**[6].**Organochlorines (OCs), including PCBs and OCPs, represent an important group of persistent organic pollutants (POPs) that have caused worldwide concern as toxic environmental contaminants. They are strongly particle associated in aquatic ecosystems due to their hydrophobic properties, and tend to accumulate in sediments **[7].** Blood is a path physiological reflector of the whole body, and therefore, blood parameters are important in diagnosing the structural and functional status of fish exposed to toxicants **[8] .** Changes in the biochemical blood profile indicate alterations in metabolism and biochemical processes of the organism, resulting from the effects of various pollutants, and they make it possible to study the mechanisms of the effects of these pollutants **[9].** Bioaccumulation is the net build-up of substances from water in an aquatic organism as a result of enhanced uptake and slow elimination of such substance **[10].** As a result of Fish living in polluted waters this lead to accumulate heavy metals in their tissues. Fish muscles, in comparison to the other tissues, usually contain the lowest amount of metals. Metal distribution in different organs is time-related. Residue of metals in various organs of fish may result in structural lesions and functional failure **[11]** o other hand, The problem of pesticide residues in food chain and freshwater fish tissues has been addressed at international level through several Committees sponsored by some United Nations Organizations **[12].**

As Fish in comparison with invertebrates are more sensitive to many toxicants and a convenient test subject for indication of ecosystem health. So, The aim of the present study is to investigate the possible protective effect of Zinc Sulphate (Znso4.7H2o) in inhibiting the toxicity of Tak (*O,O-diethyl O-3,5,6-trichloro-2-pyridylphosphoro thioate) as organochlorine pesticide on the Nile tilapia (O.niloticus). This evaluative is accomplished through measurement of Zinc and Tak pesticide bioaccumulation in fish vital organs as well as some biochemical parameters.*

II. Material And Methods

2.1. Experimental Fish

Active and healthy Nile tilapia, *Oreochromis niloticus* fish with an average body weight 110 ±10.0 g and average body length (15.2 cm) were collect from a commercial farm in Shakshouk at Fayoum Governorate, Egypt (April, 2014) .Fish were apparently free from any skin lesion and parasites . Fish were kept in fiber glass tanks and provided with aerators for 15 days for acclimatization before start experiment containingdecolorized tap water (temperature 23±1 °C, PH 6.5± 0.1, oxygen content 6.8 ± 0.9). During acclimation, the fish were fed on commercial pellets (28% protein) once per day . Water were renewed every 24h with routine clearing of the aquaria, leaving no fecal matter or unconsumed food. Two days prior to application of zinc and Tak exposure, the fish were transferred from the stock tanks to glass aquaria (60L) provided with well aerated tap water.

2.1.1. *Toxicity Bioassay*

Preliminary experiments were conducted to determine the half lethal concentration after 96h(96h-LC50) for Zn and Tak. Various concentrations for Zn as ZnSO4.7H2O(12,24,48,96,192,384,768 and 1536) and Tak (1.8,3.6,7.5,15,30,60,120 and 240) were prepared in 60 L glass aquaria. Groups of fish intriplicate monner (10 fish each) were exposed for each concentration of zinc and Tak for 96h in addation to control fish group..

The half lethal concentration after 96h(96h-LC50) was calculated according to **Behrens and karber [13]** equation and was found to be 96 mg/l for Zn and 30 mg/L for Tak.

2.1.2. Experimental design

Fish were divided into one hundred and fourty seven in seven groups with three replicates as follows . Group 1 control fish group record in decolorized tap water. Group 2 :-fish exposed to (1/20 96hr LC50) of Zn. Group 3 :-fish exposed to (1/10 96hr LC50) of Zn. Group 4 :-fish exposed to (1/20 96hr LC50) of TAK. Group 5 :- fish exposed to (1/10 96hr LC50) of TAK. Group 6 :- fish exposed to mix of (1/20 96hr LC50)Zn and Tak . Group 7 :- fish exposed to mix of (1/10 96hr LC50)Zn and Tak . feeding was allowed Once daily through out the period of the experiment(15 days). Both treated and control fish were sampled for biochemical and bioaccumulation investigations after 15 days of exposure.

2.2. Biochemical Measurements

Blood samples were taken from the caudal vein of an anaesthetised fish .About 1-3 ml of blood was collected from each individual in a clean centrifuge tube and allows to clotty at room temperature . serum was then separated by centrifuge at 3000n.p.w for 15 min to be used for estimation in following. Serum glucose concentration was determined according to the method of **Trinder [14]**using reagent kit purchased from (Randox Laboratories (UK)). Serum cholesterol concentration was estimated according to the method of **Allain *et al.*[15]** using reagent kit Purchased from (Vitro Scientific Company (Egypt)). Serum triglycerides concentration was determined according to the method of **Fossati and Prencipe [16]** using reagent kit Purchased from (Vitro Scientific Company (Egypt)). Urea concentration was estimated according to **Kaplan[17]** method using reagent kits Purchased from (Diamond Diagnostic Chemical Company (Egypt)). Creatinine was determined according to **Murray *et al*.[18]** using reagent kits from(Diamond Diagnostic Chemical Company (Egypt)) .

ALT and AST activities in liver were determined according to the method of the International Federation of Clinical Chemistry; **Scientific Committee [19]** using reagent kits purchased from (Randox Laboratories Company (UK)). The test to determined serum cortisol has to be performed on the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (Including Maglumi 600,Maglumi 1000,Maglumi 1000 Plus, Maglumi 2000,Maglumi 2000 Plus,Maglumi 3000 and Maglumi 4000) **Tunn,et al [20] .**

2.3. Residual Studied Pollutants

2.3.1. Determination of Zn: after dissection of fish samples , the liver and muscles were taken and prepared for Zn analysis. Tissue samples were digested after drying **Goldberg *et al.*[21].** The level of Zn was determined using Inductively Coupled Plasma Emission Spectrometer (ICP).

2.3.2. Determination of Tak: after dissection of fish samples , the liver and muscles were taken and prepared for Tak analysis. Fish tissues were cleaned by activated florisil using elution solvent system of 50% dichloromethane, 48.5% n-hexane, and 1-5% acetonitril gradually **Mills et al.[22]** and **Mann[23].** The level of Tak was determined using The HPLC apparatus of Model Agilent series 1100 with UV detector and C_{18} stainless column (4-6 X ^{250} mm) (Merk company).

2.3. Statistical analysis:

The data were analyzed by one-way ANOVA and significant differences were determined by Duncan Waller Multiple Range Test at 5% level using SPSS Statistical Package Program **(SPSS, 2008)** 17, released version **[59].**

III. Results

3.1. Biochemical Measurements

Table 1 show that In comparison with the control ALT value, the treated fish with high concentrations of Zn has a highly significant increase in serum ALT levels ($P \leq 0.05$). However, fish exposed to Tak (low and high concentrations) had a decreased in serum ALT but , statistically not significant . When the fish exposed to the combined concentrations of the two pollutants. The ALT level was not changed significantly. On the other hand , A significant increasing was observed in AST for a group of fish exposed to low and high concentrations of Zn or Tak ($P \leq 0.05$). When the fish were exposed to combined concentrations of two pollutants their serum AST level was increased but, statistically not significant. On other hand, Comparing to the control value, a significant elevation in serum urea level of *O.niloticus* ($P \leq 0.05$) was observed with all treatments of fish exposed for 15 days to low and high concentrations of Zn or / and Tak. Also , the exposure of *O.niloticus* to either one of the two concentrations of Zn and TAK individually as well as to the combined concentrations of the two pollutants results in a significant increasing in serum creatinine levels. The level of a significant ($P \leq 0.001$) in high concentrations as compare to the control group.

As seen in table 2 comparing control data, the levels of serum glucose and cortisol were significant increasing ($P \leq 0.05$) in *O.niloticus* exposed to either one of the two concentrations of Zn or Tak as well as to the combined concentrations of the two pollutant for 15 days (Table 2). Also , From the data present in Table 2, in fish exposed to Zn and Tak individually for 15 days , it was clear that The statistically analysis revealed that *O.niloticus* exposed to two Zn concentrations had higher level of serum total cholesterol compare to control group ($P \leq 0.05$) . Similarly the fish exposed to low and high concentrations of Tak has a high concentration of serum total cholesterol.

The exposure of fish to the combined concentrations of the two pollutant results in a significant increase in serum total cholesterol level ($P \leq 0.001$) as compare to control value. As well as results in a significant increasing in serum triglycerides level ($P \leq 0.05$) as compared to the control value.

3.2. Residual Zinc and Tak in Fish Tissue

Large variation occurred in the pattern of Zn and Tak accumulation in different investigated tissue (liver and muscles) of *O.niloticus* after the exposure to sublethal concentrations of Zn or Tak(1/20LC50 and 1/10LC50) for 15 days (Table 3). Zinc level in the studied tissues of fish exposed to two different sublethal concentrations of Zn was increased by increasing exposure concentrations. Organ-wise distribution of residual Zn revealed that the liver is the prine site of accumulation , which followed by muscle in the studied fish . similarly, after 15 days of exposure to two different sublethal concentrations of Tak was increased by increasing exposure concentrations. The accumulation of Tak in the studied tissue of *O.niloticus* was higher in liver than muscle.

3.3. Tables

Table 1 : Serum liver and kidney functions of *O .niloticus* exposed to sub lethal concentrations of Zn and Tak pesticide individually and in mixture for 15 days .

Studied groups	AST(U/L)	ALT(U/L)	Urea (mg/dl)	Creatinine (mg/dl)
Group I: Control group, fish reared in dechlorinated tap water	140 ± 1.5^{b}	58 ± 1.1^{c}	21 ± 0.9^{c}	0.5 ± 0.01^{c}
Group II :- Fish exposed to 4.8 mg Zn/l	200 ± 2.5^{a}	65 ± 1.4^{b}	40 ± 1.9^{b}	0.4 ± 0.01^{c}
Group III :-Fish exposed to 9.6 mg Zn/l	207 ± 1.8^{a}	118 ± 3.4^{a}	66 ± 0.7^{a}	0.9 ± 0.02^{a}
Group IV :-Fish exposed to 1.5 mg Tak/l	178 ± 1.7^{a}	35 ± 1.2^{c}	59 ± 1.3^{a}	0.7 ± 0.02^{c}
Group V :- Fish exposed to 3 mg Tak/l	180 ± 2.2^{a}	42 ± 2.1^{b}	61 ± 1.7^{a}	0.8 ± 0.01^{a}
Group VI :- Fish exposed to mixture of 4.8 mg Zn/l and 1.5 mg Tak/l	153 ± 0.8^{a}	40 ± 1.7^{b}	43 ± 1.4^{b}	0.9 ± 0.01^{a}
Group VII :- Fish exposed to mixture of 9.6 mg Zn/l and 3 mg Tak/l	156 ± 1.7^{a}	60 ± 2.0^{a}	56 ± 1.7^{a}	0.9 ± 0.02^{a}

- Data are represented as means of 8 samples ± Sterr
- Means in the same column with the same superscripts letter are not significantly different , otherwise they do.
- Highly significant differences at ($P \leq 0.01$)

Table2: Serum glucose , cortisol , cholesterol and triglycerides levels of *O.niloticus* exposed to sublethal concentrations of Zn and Tak pesticide individually and in mixture for 15 days .

Studied groups	Glucose (mg/dl)	Cortisol (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Group I: Control group, fish reared in dechlorinated tap water	43 ± 0.8 [d]	0.6 ± 0.01[c]	134 ± 1.7[c]	130 ± 1.4[d]
Group II :- Fish exposed to 4.8 mg Zn/l	136 ± 0. 9 [b]	0.8 ± 0.03 [b]	152 ± 1.7 [b]	295 ± 1.7 [b]
Group III :-Fish exposed to 9.6 mg Zn/l	341 ± 3.6 [a]	0.9 ± 0.02 [a]	308 ± 2.3 [a]	397 ± 2.4 [a]
Group IV :-Fish exposed to 1.5 mg Tak/l	139 ± 0.9 [b]	0.8 ± 0.02 [a]	161 ± 0.9 [b]	179 ± 2.1[c]
Group V :- Fish exposed to 3 mg Tak/l	233 ± 1.2 [a]	0.9 ± 0.02 [a]	183 ± 1.1 [b]	219 ± 2.5[b]
Group VI :- Fish exposed to mixture of 4.8 mg Zn/l and 1.5 mg Tak/l	210 ± 1.3 [b]	0.8 ± 0.02 [b]	197 ± 2.3 [b]	175 ± 2.5 [c]
Group VII :- Fish exposed to mixture of 9.6 mg Zn/l and 3 mg Tak/l	281±1.7 [a]	0.9 ± 0.02 [a]	214 ± 2.2 [a]	250 ± 1.8 [b]

- Data are represented as means of 8samples ± Sterr.
- means in the same column with the same superscripts letter are not significantly different , otherwise they do.
- Highly significant differences at ($P \leq 0.05$) .

Table 3: Residual zinc and Tak pesticide in liver and muscles (mg/kg dry weight) of *O.niloticus* exposed to sublethal concentrations of zinc and Tak pesticide individually and in mixture for 15 days.

Studied groups	Residual Zinc		Residual Tak pesticide	
	Liver	Muscles	Liver	Muscles
Group I: Control group, fish reared in dechlorinated tap water	35.40 ± 2.5[d]	10.60 ± 0.99[b]	N.D.	N.D.
Group II :- Fish exposed to 4.8 mg Zn/l	91 ± 5.5[c]	9 ± 1.3[b]	N.D.	N.D.
Group III :-Fish exposed to 9.6 mg Zn/l	117±1.6 [b]	13 ± 2.8[a]	N.D.	N.D.
Group IV :-Fish exposed to 1.5 mg Tak/l	37.40 ± 1.9[d]	9.20 ± 1.1[b]	0.51±0.002 [a]	0.02±0.001[a]
Group V :- Fish exposed to 3 mg Tak/l	33.40 ± 2.1[d]	8.40 ± 2.5[b]	0.58±0.002[a]	0.03±0.002[a]
Group VI :- Fish exposed to mixture of 4.8 mg Zn/l and 1.5 mg Tak/l	111 ± 1.41[b]	4.95 ± 0.92 [d]	0.285 ± 0.021[b]	0.025 ± 0.007 [a]
Group VII :- Fish exposed to mixture of 9.6 mg Zn/l and 3 mg Tak/l	126 ± 1.44 [a]	7 ± 1.69 [c]	0.335 ± 0.007[b]	0.015 ± 0.005 [a]

- Data are represented as means of 8 samples ± Sterr
- Means in the same column with the same superscripts letter are not significantly different, otherwise they do.
- Highly significant differences at ($P \leq 0.01$) N.D. : Not Detectable

IV. Discussion

Biochemical analysis can provide valuable information for monitoring the health conditions of fishes. Biochemical changes depend on the fish species age, the cycle of sexual maturity and health condition. Moreover, analyses of serum biochemical constituents' levels have shown useful information in detection and diagnosis of metabolic disturbances and diseases in fishes **[24].**

Blood is the most important fluid in animals reflecting the physiological condition; the blood study is now days widely used to identify the toxic impact of pollutants **[25].** Determination of glucose concentration in blood serum is widely used as an indication of stress response. Generally, glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in the plasma. In many fish species, the blood glucose level has the tendency to increase due to experimental stress. Blood glucose is caused by disorders in carbohydrate metabolism appearing in the condition of physical and chemical stresses **[26].**

The present study showed that the exposure of *O.niloticus* to sub lethal concentrations of Zn and TAK individually or in mixture as well as to the combined concentrations results in a significant increasing in serum glucose level ($P \leq 0.05$) as compared to the control value noticed after 15 days this similar to **Abdel-Tawwab,et al[27]** who reported that the effect of sublethal zinc (Zn) common carp, *Cyprinus carpio L.*. Fish The significant increase of blood glucose during Zn exposure periods Also**, Thamin and Nazmul[28]** stated that glucose levels increased during exposure of a fresh water fish, *Heteropneustes fossilis* at different concentration of Zinc (II) Sulphate for three days and **Mutlu, et al[29]** studied the effects of copper sulfate ($CuSO4(5\ H2O)$) on the Nile tilapia (*Oreochromis niloticus*) , their results showed that the serum glucose exhibited increasing during exposure periods(35,65,95 days) .The present study for effect of pesticide on blood glucose level agree with **Banaee [30]** who concluded that Glucose increase a general response of fish to acute pollutant effects of pesticides . Addition to **Hamed[31]** who reported that Nile tilapia were exposed to different sub lethal concentrations of malathion (1/2 and 1/4 LC50) for 15 days concentrations results in increasing blood glucose levels . As well as The present study for effect of combined pesticide and heavy metal(Zn and Tak) on blood glucose level agree with ***Sabae and Mohamed[5]*** who reported that The increase in value of glucose (hyperglycemia) observed in *Tilapia spp.* from Lake Qarun which exposure to heavy metal and pesticide and **Özgür F □raţ et al [32]** *studied that Oreochromis niloticus* exposed to a synthetic pyrethroid, cypermethrin (CYP); an essential metal, copper (Cu); and a nonessential metal, lead (Pb). Fish were exposed to 0.05 μg/l CYP, 0.05 mg/l Cu, and 0.05 mg/l Pb for 4 and 21 days,there are increasing in blood glucose levels.

The elevation of circulating cortisol may be a function of de novo stimulation of the HPI axis in response to pesticide and metal stress (**Özgür F □raţ et al[32]**. The present study shows the exposure of *O.niloticus* to either one of the two concentration of Zn or Tak as well as to the combined concentrations of the two pollutants results in a significant increasing in serum cortisol level ($P \leq 0.05$) as compared to the control value this similar to **Ibrahim , et al [33]** who studied that salmon were treated for 6 weeks in freshwater with 0, 200, and 400 ppb zinc (as Zn SO4). Zinc treatment resulted in some physiological changes consistent with increased plasma cortisol levels and ,**Gupta,et al [34]** reported that when *Garra gotyla gotyla* fish(three groups) exposure for three doses of manganese as heavy metal for 1,5,9th weeks there are significant increase in the levels of cortisol of three groups of fish after 1st week of exposure to three doses of manganese as well as significant decrease in the cortisol of three groups exposed for 5th & 9thweek.

On other hand, The present study for effect of pesticide on blood cortisol similar to **Hamed[31]** who reported that Nile tilapia were exposed to different sub lethal concentrations of malathion (1/2 and 1/4 LC50) for 15 days concentrations results in cortisol level was significantly increased As well as, **Benguira and Hontela[35]** stated that Exposure of fish to different types of pesticides showed alterations in the serum cortisol levels in fish such as *Oncorhynchus mykiss* to DDT compounds.The present study for effect of combined pesticide and heavy metal(Zn and Tak) on blood cortisol level similar to **Özgür F □raţ et al [32]** *studied that Oreochromis niloticus* exposed to a synthetic pyrethroid, cypermethrin (CYP); an essential metal, copper (Cu); and a nonessential metal, lead (Pb). Fish were exposed to 0.05 μg/l CYP, 0.05 mg/l Cu, and 0.05 mg/l Pb for 4 and 21 days,there are increasing in cortisol level.

The increased value of plasma total lipids, triglycerides and total cholesterol indicated retardation of fat metabolism cholesterol may be due to one or more of the following reasons ***Sabae and Mohamed[5])*** 1) increased the effect of the pollutants (heavy metals and pesticide) 2) release of cholesterol and other lipids constituents from damagedthe target organs of toxicity as well as the cell membranes. The present The statistically analysis revealed that *O.niloticus* exposed to two Zn concentrations had higher level of serum total cholesterol compare to control group ($P \leq 0.05$) . Similarly the fish exposed to low and high concentrations of Tak has a high concentration of serum total cholesterol . the exposure of fish to the combined concentrations of the two pollutant results in a significant increase in serum total cholesterol level ($P \leq 0.001$) as compare to control value noticed after 15 days exposure also ,triglycerides showed a significant increasing in serum triglycerides level ($P \leq 0.05$) as compared to the control value. This similar to **Parvathi, et al [36]**studied that The Common carp, *Cyprinus carpio* was exposed to sublethal concentrations of chromium for various exposure periods (8, 16, 24 and 32 days). Serum total cholesterol were measured both in control and experimental fish. During various exposure periods the levels of total cholesterol significantly elevated And **Heydarnejad,et al [37]**stated that the effects of cadmium at sub-lethal concentrations (1 and 3 μg/l) on growth and serum biochemical parameters of rainbow trout (Oncorhynchus mykiss) fish including Triglyceride and cholesterol and showed decreased transiently at day 15 and then increased at day 30.

Addition to ,**Bhattacharjee and Das [38]**stated that Fish exposed to lindane showed a significant increase in total lipid during exposure to acute lethal and sublethal concentrations of landan as an organochlorine pesticide. Similar total lipids, triglycerides and total cholesterol were noted by **Shalaby *et al[39]*** in *Oreochromis niloticus* exposed to butataf herbicide, And agree with **Sabae and Mohamed[5]** who reported that The increase in value of plasma total lipids, triglycerides and total cholesterol observed in Tilapia spp. from Lake Qarun which exposure to heavy metal and pesticide.

On the other hand, Creatinine is a breakdown product of Creatine Kinase in muscle. The creatinine test has been usually used to diagnose impaired kidney function and to detect renal damage in animals like fish **Banaee *et al[40]*.**Urea is present in all fish, the liver being the primary organ of production and the gills appearing to be the main as well as organ of excretion. freshwater fish excrete ammonia along with a small quantity of urea as they use urea as an osmotic filter.Urea in fish is synthesis by the liver and excreted primarily by the gills rather more the kidney. the elevation of urea level may be attributed to gill dysfunction **Stoskoph[41]** It is shown that the increased blood urea could occur at times of impaired kidney function, liver diseases and cardiac arrest **Abdelmoneim,etal [42]**Similar increase in plasma urea, uric acid and creatinine were noted by **Shalaby *et al[39]***in *Oreochromis niloticus* exposed to butataf herbicide and **El-Boshy and Taha [43]** in Nile Tilapia .

The present study showed In comparison with the control value, a significant elevation in serum urea level of *O.niloticus* ($P \leq 0.05$) was observed with all treatments of fish(1/20 lc50 and 1/10lc50) also, creatinine significant increasing noticed after 15 days exposure this agree with Mohamed and Gad[44]studied The effects of both metals zinc and/or cadmium on biochemical parameters of o.niloticus fish urea and creatinine and results showed that there are significant increasing during exposure . And , similarity with **Mutlu, et al[29**] who studied the effects of copper sulfate (CuSO4(5 H2O)) on the Nile tilapia (*Oreochromis niloticus*) , their results showed that creatinine of the exposed group significantly increased. Also , similar to **Zaghloul [45]** who reported that the effect of sublethal zinc (Zn) common carp, Cyprinus carpio L.. Fish The significant increase Uric acid and creatinine levels in fish serum increased. Addition to , **Deepak[46]** reported that the freshwater fish *Cyprinus carpio* (Linn) *was exposure to* sublethal concentration of the pesticide cypermethrin for 28 days result in increase urea and creatinine levels. And , **Orun, et al[47]** studied that *Oncorhynchus mykiss* exposed to cypermethrin (CYP). The fish were subjected to three sublethal concentrations of CYP (0.0041, 0.0082 and 0.0123 ppm) (10 ppm) for 96 h. The samples were analysed to changes in the biochemical parameters, such as the metabolits: creatinine, urea The data of this investigation showed that CYP had a negative effect on the biochemical parameters.

Generally, the results of ALT, ASTmay indicate degeneration changes and hypofunction of liver as the toxicants effects on the hepatocytes are in the form of tissue damage in which cellular enzymes are released from the cells into the blood serum. Therefore, increases in these enzyme activities in serum of *O. niloticus* is mainly due to the leakage of these enzymes from the liver cytosol into the blood stream as a result of liver damage by pesticide and metals, which gives an indication of the hepatotoxic effect of toxicants **Özgür F □raţ*et al[32].***

The present study showed that In comparison with the control ALT value, the treated fish with high concentrations of Zn has a highly significant increase in serum ALT levels ($P \leq 0.05$). However, fish exposed to Tak (low and high concentrations) had a decreased in serum ALT but , statistically not significant . When the fish exposed to the combined concentrations of the two pollutants. The ALT level was not changed significantly. This agree with **Younis,et al[48]** studied that the activites of ALT and AST were increased significant in *O.niloticus* after exposure to 0.25,0.50 and 0.75 LC50of Zn after 4 weeks. Addition to , **El Sayed[49]** reported that Some biochemical changes resulting from the exposure of Nile Tilapia to sub lethal concentrations (15, 30 and 45 mg L-1) of zinc sulfate in water for a period of 24 hr, 48 and 72hr. The results indicate serum AST and ALT activities also, **Firat and Kargin [50]**found increases in ALTand ASTactivity in Nile tilapia serum caused by the individual and combined effects of exposure to Zn and Cd noticed after 56 days. As well as , **Ayyat,et al [51]** studied the investigation of the influences of sublethal toxicity of Isoprothiolan (Fuji-one)on Oreochromis niloticus. evaluated on the 60th day of exposure to 1/12 1/6 and 1/3 LC50 of the pesticide in comparison with control fish group.,The plasma Aspartate amino-transferase (AST) and Alanine amino-transferase (ALT) enzymes activities were elevated significantly in fish exposed to Fuji-one. Addition to , **Saravanan, et al [52]**studied that The major carp, Labeo rohita (Hamilton) upon chronic exposure to endosulfan showed the tissue metabolites and enzymes like AST and ALT revealed significant alterations. similar to **Özgür F □raţ et al [32]**who *studied that Oreochromis niloticus* exposed to a synthetic pyrethroid, cypermethrin (CYP); an essential metal, copper (Cu); and a nonessential metal, lead (Pb). Fish were exposed to 0.05 μg/l CYP, 0.05 mg/l Cu, and 0.05 mg/l Pb for 4 and 21 days, and the alterations in serum enzyme activities, Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities increased in response to CYP, Cu, and Pb exposures at both exposure periods.

It is generally metal accumulation in tissues of aquatic animals is dependent upon the exposure concentration and period, as well as some factors, such as salinity, temperature, interacting agents and metabolic activities of tissue concerned The mobility as well as the toxicity of zinc to aquatic species is enhanced by the physicochemical characteristics of inhabiting medium, such as temperature, hardness, and pH **Shukla et al[53]**. On the other hand, **Kaoud [54]**reported that heavy metals and pesticides have begun to raise a public attention in water and aquaculture ecosystems, contribute to the larger problem of aquatic pollution, which can seriously damage the marine environment and cause health hazards to people in some areas. Moreover, increasing pollution of groundwater and surface water from a wide variety of industrial, municipal, and agricultural sources has additionally seriously tainted water quality in these sources, effectively reducing the supply of freshwater for human use, considered factors affect on rate of accumulation of these pollutants.

The present study focused on Concentration of Zn in mg/Kg dry wt (ppn) or Tak in mg/Kg dry wt (ppn) accumulation in liver and muscles of *O.niloticus* after 15 days of exposure. There are significant increasing was found in the level of accumulation in liver of *o.niloticus* than muscles. The present study for bioaccumulation of heavy metal (Zn) agree with **Mohamed and Gad[5]** stated that the bioaccumulation of zinc and cadmium in the liver, gills and muscle of Oreochromis niloticus after 15 days of exposure to sublethal concentrations of Zn and/or Cd were investigated and results showed that accumulation of Zn and/or cadmium increasing with increasing the exposure period and the accumulation rate is liver > gills>muscles. And similar to

, **Kumar,et al[6]** stated that bioaccumulation of Zn in fresh water fish *Channa punctatus in some organ* liver, kidney, gill, muscle, testis and ovary after the different intervals of exposure periods 7 days, 14 days, 21 days and 28 days.

Bioaccumulation was comparatively higher in liver and kidney than other organs addation to, **Ibemenuga and Nwamaka[55]**The contamination of the aquatic systems with heavy metals from natural anthropogenic sources has become a global problem which poses threats to ecosystems and natural communities, Hence this study reviews the effects of heavy metals in freshwater fishes. Fishes bioaccumulate heavy metals (including cadmium, zinc, lead and copper) through various organs such as gills, liver, stomach and intestine. The effects of these heavy metals are highlihted.

On the other hand, The present study for bioaccumulation of pesticide (Tak) similar to **EL-Nemkai,et al [56]**reported that The values of different pesticide in fish muscle from ponds that received irrigation water were higher compared to those that received agriculture drainage water showed that the concentrations of most pesticides were at their highest level in the fish gills from the irrigation water, followed by the fish liver and, **ABDUL [57]**who reported that the levels of major pesticides and their bioaccumulation in fish and shrimp river shad (*Tenualosailisha*), silver jewfish (*Otolithoides pama*), long whiskers catfish (*Mystus gulio*) and Gangetic hairfin anchovy (*Setipinna phasa*) and one benthic invertebrate: black tiger shrimp (*Penaeus monodon*) and were analyzed for four pesticides like heptachlor, diendrin, endrin and DDT. In general, the liver exhibited higher levels of pesticide concentrations than the muscle also, **Bhuvaneshwari ,et al [58]** Their study monitored the toxic effect of environmental concentration of pesticides and metals in zebra fish Danio rerio. Zebra fish were insecure to these toxic pollutants for a period of 14 days. The individual and the combined toxic effect of pesticides and metals were evaluated. Significant alterations were showed in liver and gill comparing to muscle tissue.

V. Conclusion

Most important conclusion from these studies is that the toxicity exists and that Zinc and Tak pesticide combinations may exert their determinal effects equitoxic mixtures even at very low concentrations. Moreover, the interaction of toxicity is an important factor which must be taken into consideration when assessing hazards of environmental pollutants to aquatic life and for setting valid water quality standards for diverse uses.

References

[1]. Jaya Sharma, Pollution-a grave threat to the environment, man kind and the living world, *Pacific Business Review International 8, 2016,pp164.*

[2]. Chandran.R, Sivakumar. A.A, Mohandass.S, and Aruchami. M, Effect of cadmium and zinc on antioxidant enzyme activity in the gastropod, *Achatina fulica*. *Comp Biochem Physiol.140,2005,422:426.*

[3]. Dutta. H.M, and Dalal.R, The effect of endosulfan on the ovary of bluegill sunfish: a histopathological study (*Lepomis macrochirus* sp*), International Journal Environ Res. 2, 2008,215-224.*

[4]. WHO/FAO/IAEA. World Health Organization. Switzerland: Geneva(1996). Trace Elements in Human Nutrition and Health.

[5]. Shawky. Z. Sabae, and Fatma. A.S. Mohamed, Effect of Environmental Pollution on the Health of Tilapia spp. from Lake Qarun, *Global Veterinaria 14 (3),2015, 304-328.*

[6]. Manoj Kumar, Arun Ratna, Rajesh Prashad, S.P.Trivedi, Y.K.Sharma, and A.K.Shukla, assessment of zinc bioaccumulation in fish *channa punctayus* exposed chronically, *G.J.B.B., 4 (4) ,2015,347-355.*

[7]. Binelli. A, Sarkar. S.K, Chatterjee.M, Riva. C, Parolini M , Bhattacharya. B.D, A comparisonof sediment quality guidelines for toxicity assessment in the Sunderban wetlands (Bay of Bengal, India), *Chemosphere 73,2008,29-37.*

[8]. Adhikari.S, Sarkar. B, Chatterjee.A,Mahapatra. C.T, and Ayyappan.S, Effects of cypermethrin and carbofuran on certain hematological parameters and prediction of their recoveryin a freshwater teleost, *Labeo rohita*(Hamilton). *Ecotoxicology and Environmental Safety, 58,2004, 220-226.*

[9]. Luskova.V, Svoboda.M, and Kolarova. J ,The effects of diazinon on blood plasma biochemistry in carp (*Cyprinus carpio* L.), *Acta Vet Brno. 71,2002,117-123.*

[10]. Bhattacharya.A.K, S.N. Mandal, and S.K. Das, Heavy metals accumulation in water, sediment and tissues of different edible fishes in upper stretch of Gangetic West Bengal,*Trends Applied Sci. Res., 3, 2008,61-68.*

[11]. Barbara Jezierska, and Małgorzata Witeska, the metal uptake and accumlation in fish living in polluted waters Publisher Springer Netherlands, *69 Soil and water pollution monitoring protection and Remedation 2006,107-114.*

[12]. Hashim. M.F, and Salem.G.S, Studies on some pesticide residues in cultured and wild fish, *J. Egypt. Vet. Med. Assoc. 66 (4),2006 249 -258*.

[13]. Behrens and Karber, Determination of LD50 , *Arch.Fur.Exp.path., and Pharm ,1953, 177-372.*

[14]. Trinder.P, Determination of glucose inblood using glucose oxidase with an alternative oxygen receptor, *Ann. Clin. Biochem. 6,1969, 24-27.*

[15]. Allain C. C, Poon .I. S, Chan. C. H. G, Richmond. W, and Fu .P. C , Enzymatic determination of serum total cholesterol, *Clin. Chem. 20,1974,470-471.*

[16]. Fossati. P, and Prencipe. L,Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clinical Chemistry 28(10),1982, 2077-2080.*

[17]. Kaplan. A, Urea. Kaplan A et al, *Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton, 1984,1257-1260 and 437 and 418.*

[18]. Murray, R. Aspartate aminotransferase. Kaplan A et al.,*Clin Chem, The C.V. Mosby Co. StLouis. Toronto. Princeton, 1,1984,112-116.*

[19]. Scientific Committee Expert Panel on Enzymes, *J. Clin. Chem. Clin. Bio ehe m. 18 ,1980,521-534.*

[20]. Tunn. S, Pappert. G,Willnow. P, Krieg. M, Multicentre evaluation of an enzyme immunoassay for cortisol determination, *J Clin Chem Clin Biochem .28,1990,929-935.*

[21]. Goldberg.E.D, Geochronologywithlead-210. In: Symposium on radioactive dating, Athens, November, 1962. Vienna, *InternationalAtomic Energy Agency. 1963,121:131.*

[22]. Mills. P. A, A. B. Baraka, R. K. L. Verene, and A. B. Jerry, Elution solvent systems for florisil clean up in organochlorine pesticide residues analysis, *J.A.O.A.C, 5,1972,39-43.*

[23]. Mann. B. J, Manual for training of pesticides analysis. USA. *Section 11-B,1981, 2-5.*

[24]. Ferrari. A, Venturino.A, and Pechén. A.M.de D'Angelo, (2007) Effects of carbaryl and azinphos methyl on juvenile rainbow trout (Oncorhynchus mykiss) detoxifying enzymes.,*88(2), Pesticide Biochemistry and Physiology,2007,134-142.*

[25]. Lakshmanan. S.A, and Rajendran.C. Sivasubramaniyan,Impact of Dichlorvos on tissue glycogen and protein content in freshwater fingerlings, *Oreochromis mossambicus* (Peters). *International Journal of Research Journal of Entomology and Zoology Studies in Environmental Science and Technology 3(1),2013,19-25*

[26]. Parikh.P.H , pyrazosulforun-ethyl induced alternation in hematology and blood biochemical on *oreochromis mossambicus:* sub-acute study the experiment 18(3),2014,1245-1253.

[27]. Mohsen Abdel-Tawwab, Mohamed. N.M. Mousaad, Khaled. M. Sharafeldin, and Nahla. E.M .Ismaiel, Changes in growth and biochemical status of common carp, Cyprinus carpio L. exposed to water-born zinc toxicity for different periods. *International Aquatic Research,2013, 5-11.*

[28]. Tahmina Hoq, and Nazmul Haque, Effects Of Sublethal Concentrations Of Zinc (II) Sulphate Heptahydrate (Znso4.7H2O) On Blood Glucose Level Of Freshwater Fish Heteropneustes Fossilis. *International journal of scientific and technology research 3,2014, 2277-8616.*

[29]. Ekrem Mutlu, Seyit Aydın, and Banu Kutlu , Alterations of Growth Performance and Blood Chemistry in Nile Tilapia (*Oreochromis Nuoticus*) Affected by Copper Sulfate in Long-Term Exposure, *Turkish Journal of Fisheries and Aquatic Sciences, 15,2015, 481-488.*

[30]. Banaee. M, Adverse effect of insecticides on various aspects of fish's biology and physiology: Insecticides- Basic and Other Applications Book, Edited by Sonia Soloneski and Marcelo Larramendy, *In.Tech. 6,2012, 101-126.*

[31]. Heba. S. Hamed,Impact of a Short – Term Malathion Exposure of Nile Tilapia, (*Oreochromis niloticus*): The Protective Role of Selenium, *International Journal of Environmental Monitoring and Analysis. Special Issue: New Horizons in Environmental Science 3,2015,30-37.*

[32]. Özgür Fırat, Hikmet, Y. Cogun, Tüzin. A, Yüzereroğlu, Gülbin Gök, Özge Fırat, Ferit Kargin, and Yasemin Kötemen, A comparative study on the effects of a pesticide (cypermethrin) and two metals (copper, lead) to serum biochemistry of Nile tilapia, *Oreochromis niloticus, Fish Physiol Biochem 37(3),2011, 657-666.*

[33]. Ibrahim.B.M, MacKinnon, and M.D.B. Burt, The influence of sub-lethal levels of zinc on smoltifying Atlantic salmon Salmo salar and on their subsequent susceptibility to infection with Lepeophtheirus salmonis, *Contributions to Zoology, 69 (1/2),2000, 119-128*

[34]. Kadambri Gupta, Seema Langer, Jyoti Sharma, and Sheetal Sharma,Effect of different sublethal concentrations of Manganese on the levels of cortisol in *Garra gotyla gotyla International Journal of Scientific and Research Publications 2, 2012, 2250-3153.*

[35]. Benguira.S,and A. Hontela,(2000). Adrenocorticotrophin and cyclic adenosine 3′,5′-monophosphate-stimulated cortisol secretion in interrenal tissue of rainbow trout exposed in vitro to DDT compounds. *Environ. Toxicol. Chem., 19,2000, 842-847.*

[36]. Kumar Parvathi, Palanivel Sivakumar, Mathan Ramesh, and Sarasu, sublethal effects of chromium on some biochemical profiles of the fresh water teleost , *Cyprinus carpio. International Journal of Applied Biology and Pharmaceutical Technolog 2,2011, 295-300.*

[37]. M. Saeed Heydarnejad, Mozhdeh Khosravian-Hemamai, and Amin Nematollahi, Effects of cadmium at sub-lethal concentration on growth and biochemical parameters in rainbow trout (*Oncorhynchus mykiss*), *Ir Vet J. 66(1),2013, 11.*

[38]. Debasish Bhattacharjee, and Suchismita Das, Toxicity of organochlorine pesticide, Lindane to fish: *A review Journal of Chemical and Pharmaceutical Research,5(4),2013,90-96.*

[39]. Shalaby.A, M. Mousa, and H. Tag El-Dian,effect of butataf herbicide on some physiological aspects and the reproductiveNile Tilapia *Oreochromis niloticus*. *Egyptian Journal of Aquatic Biology and Fisheries, 11(2),2007, 145-163.*

[40]. Banaee. M, Sureda. A, Mirvaghefi. A. R, and Ahmadi. K, Effects of diazinon on biochemical parameters of blood in rainbow trout (*Oncorhynchus mykiss*), *Pesticide Biochemistry and Physiology 99,2011,1-6.*

[41]. Stoskoph. M, (1993)Fish Medicine. *W.B. Saunders Company, 116,1993,128-129.*

[42]. Abdelmoneim. A, N. AbouShabana, S. Khadre, and K. Abdel, Physiological and histopathological effects in cat fish (*Clarias lazera*) exposed to dyestuff and chemical water,*International Journal of Zoological Research, 4(4),2008, 189-202.*

[43]. El-Boshy. M, and R. Taha, Effects of mercuric chloride on the immunological, hematological, biochemical parameters and diseases resistance of Nile Tilapia challenged with *Aeromnas hydrophila*. *Nature and Science, 9(12),2011, 7-15.*

[44]. Mohamed. F.A.S, and N.S. Gad, Bioaccumulation, some blood biochemical changes and histological alterations in selected tissues of *Oreochromis niloticus* exposed to zinc and/or cadmium. *American-Eurasian Journal of Agricultural and Environmental Sciences, 5(3),2009, 441-455.*

[45]. Zaghloul. K.H, Usage zinc and calcium inhibiting the toxicity effect of copper on the African catfish (Clarias gariepinus). *J-Eghypt-Ger.SOC.ZOOL.,35(c), 2001,99-120.*

[46]. Paritha Bhanu. A, and Deepak.M, Impact of cypermethrin on biochemical aspects of clinical importance in the blood of freshwater fish *Cyprinus carpio Journal of Entomology and Zoology Studies . 3 (1),2015, 126-128.*

[47]. Orun, Selamoglu Talas, Z.Gulhan, and M. F.Er,dogan.K, Role of propolis on biochemical and hematological parameters of *Oncorhynchus mykiss* exposed to cypermethrin, *Journal of Survey in Fisheries Sciences 1(1),2014,21-35.*

[48]. Younis.E. M, Abdel-Warith. A. A, and Al-Asgah. N. A, Hematological and enzymatic responses of Nile tilapia *Oreochromis niloticus* during short and long term sublethal exposure to zinc, *African Journal of Biotechnology 11(19),2012,4442-4446.*

[49]. El Sayed A. M. Shokr, Effect of zinc on hematology and biochemistry of Nile Tilapia, *Journal of Chemical and Pharmaceutical Research, 7(3),2015, 1943-1950.*

[50]. Firat. O , and Kargin .F, Individual and combined effects of heavy metals on serum biochemistry of Nile tilapia, Oreochromis niloticus, *Arch Environ Contam Toxicol 58,2010,151-157.*

[51]. Ayyat.M.S.A, Shalaby. A.M , Abd-El-Rahman.G.F, and Al-Zahaby .M.A, (2014) hematological and biochemical changes in female Oreochromis niloticus after exposure to Fuji-one fungicide , *Conference of Central Laboratory for Aquaculture Research,2014,333-350 .*

[52]. T.S. Saravanan, P. Rajesh, and M. Sundaramoorth, Studies on effects of chronic exposure of endosulfan to Labeo rohita .*Journal of Environmental Biology 31(5) ,2010,755-758 .*

[53]. Shukla. V, Dhankhar.M, Prakash.J, and Sastry. K.V, Bioaccumulation of Zn, Cu and Cd in Channa punctatus.,Journal of Environmental Biology, 28 (2),2007, 130-159.

[54]. Kaoud Hussein. A, Article review: Heavy metals and Pesticides in Aquaculture: Health problems , *European Journal of Academic Essays 2(9),2015, 15-22.*

[55]. Ibemenuga, and Keziah Nwamaka, bioaccumulation and toxic effects of some heavy metals in fresh water fishes, Animal Research International 10(3),2013,1792 -1798.

[56]. Fatma El-nemaki, Nema A. Ali, Mohmed. M. Zeinhom, and Olfat, A. Radwan,(2008) impacts of different water resources ON on the ecological parameters and the quality of Tilipia production El-abbassa fish farms in egypt. *International Symposium on Tilapia in Aquaculture 2008, 491.*

[57]. M.D. ABDUL AHAD, Bioaccumulation of organochlorine pesticide in fish and potential health risk assessment from the river karaniphuli and the rupsa,2014, 346.

[58]. Bhuvaneshwari. R, Padmanaban.K, and BabuRajendran. R, Histopathological alterations in muscle, Liver and gill Tissues of Zebra Fish

[59]. Danio Rerio due to environmental relevant concentrations of organochlorine Pesticides (OCPs) and heavy metals. *Int. J. Environ. Res., 9(4),2015,1365-1372.*

[60]. SPSS. (2008). Statistical Package For Social Science (for Windows). Release 17 Copyright (C), SPSS Inc., Chicago, USA.